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A new species of *Andrena* (Hymenoptera, Andrenidae) from northern Sardinia (Italy)

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Abstract

In this paper, a new species belonging to the *Andrena pilipes/nigrospina* complex, *Andrena culucciae* **sp. nov.**, is described and illustrated from northern Sardinia (Italy). The species is distinguished by significant morphological characters, including the structure of the genital capsule and the translucent marginal zones of the tergites in males, as well as the coloration patterns of setae in females. These characters are discussed in detail and compared with those of closely related species. Morphological evidence is complemented by COI barcode data, which supports the distinctiveness of the new taxon, although they do not fully resolve the phylogenetic relationships within the *A. pilipes/nigrospina* complex. Both Neighbor-Joining and Maximum Likelihood trees consistently place *A. culucciae* **sp. nov.** in a well-supported monophyletic clade. The COI data also reveal p-distance values between *A. culucciae* **sp. nov.** and both *A. nigrospina* Thomson, 1872 and *A. pilipes* Fabricius, 1781 that are higher than those observed between *A. nigrospina* and *A. pilipes* themselves, and show distinct haplotypes for all three taxa. Furthermore, we argue that the Sardinian subspecies *Andrena pilipes iliensis* Alfken, 1938 is not valid, as it lacks significant morphological and molecular divergence from *A. pilipes* s.s. An identification key for the *Andrena pilipes/nigrospina* complex is provided. Ecological data on *A. culucciae* **sp. nov.** are also presented, suggesting a possible association with coastal dune habitats. This highlights important conservation implications, given the ecological vulnerability of these environments in the Mediterranean region.

Keywords

Andrena culucciae, Apoidea Anthophila, Culuccia Peninsula, DNA barcoding, Mediterranean, molecular analysis, Scanning Electron Microscope, synonym, wild bees

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Introduction

Wild bees are one of the most important and diverse groups of pollinators, with over 20,000 species worldwide (Michener 2007). Italy hosts a rich bee fauna, with approximately 1000 species (Lhomme et al. 2020; Reverté and Miličić et al. 2023), as it lies within the Mediterranean basin, a recognized global hotspot for bee diversity (Orr et al. 2021). Although knowledge of wild bees in Italy is incomplete, recent studies are improving our understanding of their local distribution at the national level (e.g. Cornalba et al. 2024; Fortini et al. 2024; Goglia et al. 2024; Zanini et al. 2025). In particular, new records and species for science have recently been reported from Sardinia (e.g. Catania et al. 2021; Nobile et al. 2021; Praz et al. 2022; Annessi et al. 2025a), the second largest island in the Mediterranean, which hosts many distinctive species, including endemic taxa shared with Corsica or other regions, as well as several species with a very restricted distributions (Grill et al. 2007).

The genus *Andrena* Fabricius, 1775 is a very species-rich genus that currently contains approximately 1,738 species worldwide (Wood 2025), divided into 101 subgenera according to Dubitzky et al. (2010), but a recent revised classification of Pisanty et al. (2022) suggested around 130 subgenera. The count of species known from Italy has fluctuated over time, with 173 species listed by Pagliano (1994), 234 by Comba (2019) and 218 by Wood et al. (2023). Within *Andrena*, the subgenus *Plastandrena*, established by Hedicke (1933), comprises 33 valid species worldwide according to Michener (2007), to which three additional species previously assigned to the subgenus *Agandrena*, recently synonymised with *Plastandrena* (Pisanty et al. 2022), should now be added. *Plastandrena* is mainly recognisable by the hind tibial spur, which is clearly broadened at its base combined with the rugose areolate propodeal triangle, rounded pronotum, black male face (never with yellow maculations), and coarsely (not minutely and finely) punctate terga (Pisanty et al. 2022).

Among species of this subgenus, the taxonomic status of the *Andrena pilipes/nigrospina* complex is confused and has been extensively debated (Wood 2023a). Since the 19th century, several authors have described some forms in this complex from different geographical areas under different names, which have been synonymised with two closely related species: *Andrena pilipes* Fabricius, 1781 and *Andrena nigrospina* Thomson, 1872 (see Baker 1994, 2000; Schmid-Egger and Patiny 1997; Gusenleitner and Schwarz 2002). The former is a bivoltine taxon (March-April; July-August) with narrow penis valves, whereas the latter is a univoltine taxon (May-June) with basally broad penis valves (Schmid-Egger and Patiny 1997; Wood 2023a). According to Baker (2000) and Wood (2023a), the species can only be distinguished by examining the genital capsule of the male, whereas female specimens cannot be reliably distinguished morphologically due to variations in the colour of the pubescence between generations or species. The genetic separation of these two species has been challenging due to the lack of confidently identified material (Schmidt et al. 2015). However, Wood (2023a,b) found genetic support for these taxa by the analysis of new genetic sequences from different geographic regions and from phenological data: each clade showed low

intraspecific variation (0.00–0.37% for *A. pilipes*, 0.00–1.06% for *A. nigrospina*), while variation between the two clades ranged from 1.06% to 2.35%, with an average of 1.63%. Although the overall absolute range extent of these two taxa is not fully defined, *A. nigrospina* has a distribution through more temperate regions and is found in Austria, Belgium, France, Finland, Germany, Hungary, Ireland, Kyrgyzstan, Poland, Turkey, Spain and the United Kingdom, while *A. pilipes* is more thermophilic and has a more southerly distribution, dominating in the Mediterranean basin (including Italy) and extending into Central Asia to China (Wood 2023a). In addition, Alfken (1938) described the subspecies *Andrena pilipes iliensis* from Sardinia, characterized by small size, black scopa and more finely punctate mesonotum and terga. However, the existence of this subspecies is controversial, as it appears to be a morphological variation induced by the climatic conditions of southern regions, since individuals with black scopa have also been found in Corsica, Sicily, Morocco, southern Greece, and Crete (Grünwaldt 1976; Baker 1994; Schmid-Egger and Patiny 1997).

During a recent survey of flower visiting insects on the Culuccia peninsula (northern Sardinia, Italy) (Annessi et al. 2025a,b), we found a new *Andrena* species of the *A. pilipes/nigrospina* complex, which shows morphological and molecular differences from the currently known related taxa. In this paper, we describe this new taxon and discuss its affinities with the closest species. Moreover, we suggest that the Sardinian subspecies *Andrena pilipes iliensis* is not valid, as it shows no significant morphological and molecular differences from *A. pilipes* s.s.

Materials and methods

Field observations on *Andrena culucciae* sp. nov. were carried out in the Culuccia Peninsula (northern Sardinia) between May and June 2022–2024 (Fig. 1). The peninsula, which covers approximately 300 hectares, is situated at an altitude of 0–93 m above sea level and is mainly characterized by Mediterranean scrub, followed by transitional woodland/shrub and a mosaic of other environments including wetlands, herbaceous dunes, bare rock, pasture, garigue, beach and vineyards (Annessi et al. 2025b). Bees were collected using nets between 10 am and 4 pm. Each individual was assigned a unique identification code, collection date, WGS84 coordinates, environmental type and the plant species on which it was observed. Specimens were euthanized by freezing at –20 °C for subsequent DNA analysis, then stored dry in entomological boxes and preserved at the Museum of Zoology and Comparative Anatomy, Department of Science, Roma Tre University (Rome, Italy).

Total genomic DNA was extracted from one leg of eight specimens (one male and seven females) using the salting out protocol (Sambrook et al. 1989), eluted in 100 µL H_2O milliq and stored at -20 °C. The barcode fragment of mitochondrial Cytochrome C Oxidase subunit I gene was amplified using the primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5' TAAACTTCAGGTGACCAAAAATCA-3') (Folmer et al. 1994). For each PCR, an aliquot of 1 µL of DNA and

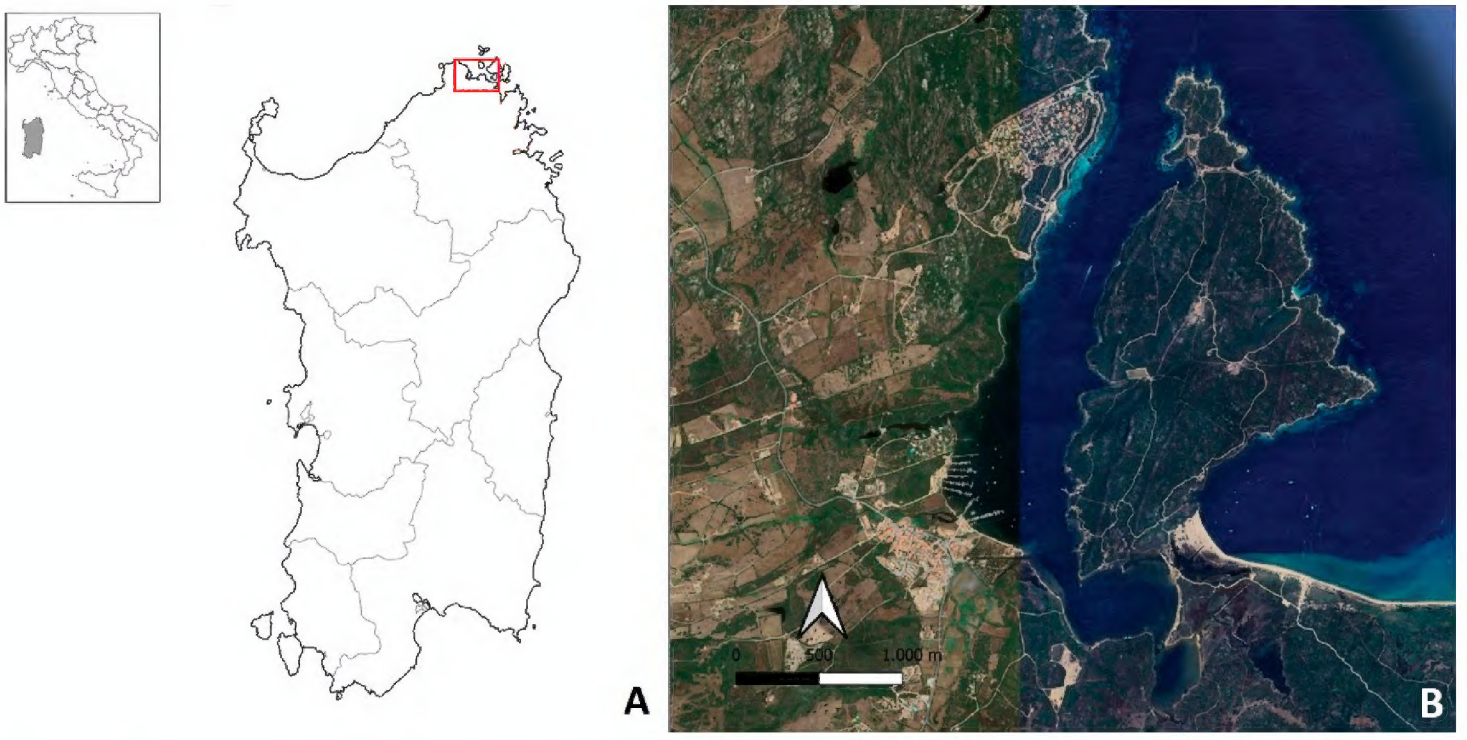


Figure 1. Position of the study area in Sardinia and in Italy (**A**). Satellite image of the Culuccia Peninsula (**B**), base map: orthophoto © 2024 Google.

24 µL of reaction mixture (MIX) were used. The MIX contained: 3 µl of Buffer 10×, 1 µl MgCl2 50 mM, 0.5 µl dNTPs 10 mM, 0.5 µl primer forward 10 mM, 0.5 µl primer reverse 10 mM, 0.5 µl BSA, 0.2 Taq DNA polymerase 5U/µl and 17.8 H2O milliQ. The PCR cycling conditions were based on Marconi et al. (2022). To verify amplification success, 3 µL of PCR product were run on a 1% agarose gel stained with 1 µL of Midori Green (Nippon Genetics Europe). Successfully amplified products were then purified and sequenced by Macrogen (Milan, Italy).

COI sequences were edited with Geneious Prime ver. 2023.1.2 (https://www.geneious.com) and aligned using MUSCLE (Edgar 2004) in MEGA 12 (Kumar et al. 2024), together with sequences of other *Plastandrena* species from the Western Palaearctic and three sequences each of *Andrena flavipes* Panzer, 1799, *Andrena thoracica* (Fabricius, 1775), *Panurgus corsicus* Warncke, 1972 and *Panurgus canescens* Latreille, 1811, used as outgroups, all downloaded from BOLD (Suppl. material 1). The resulting alignment was used to build a consensus Neighbor-Joining (NJ) phylogenetic tree in MEGA 12 (1000 bootstrap replicates (BS); K2P model) and a Maximum Likelihood (ML) tree with IQ-TREE web (Trifinopoulos et al. 2016). For ML inference, the best substitution model was selected with ModelFinder (Kalyaanamoorthy et al. 2017) and the tree search was carried out setting the number of unsuccessful iterations to 1000. Node support was estimated by applying 1000 replicates of ultrafast bootstrap (UFBboot2; Hoang et al. 2018) and Shimodaira-Hasegawa (SH)-like approximate ratio tests (SH-aLRT; Guindon et al. 2010). To explore the genetic diversity within the *pilipes*/*nigrospina* species complex a distance matrix for the species *A. pilipes*, *A. nigrospina* and *A. culucciae* sp. nov. was built with MEGA 12 using the p-distance model (Srivathsan and Meier 2012) and a haplotype network was

produced using TCS v 1.21 (Clement et al. 2000). The network was visualized in TcsBU (Múrias dos Santos et al. 2016). The *Andrena culucciae* sp. nov. sequences were deposited in BOLD and GenBank under Acc. n° PX058884–PX058891.

To morphologically compare the new taxon with the closely related taxa, comprehensive data from the literature was consulted (e.g. Baker 1994; Schmid-Egger and Patiny 1997; Gusenleitner and Schwarz 2002; Wood 2023a,b). In addition, material from the wild bee collections deposited at the "Museum of Zoology and Comparative Anatomy" of Roma Tre University (Rome, Italy) and at the "Museum of Zoology" of La Sapienza University of Rome (Rome, Italy), was examined (Table 1). Acronyms for the collectors, identifiers and depositories are as follows: **ADG** Andrea Di Giulio; **AR** Alessandra Riccieri; **DL** Diana Lucente; **ER** Enrico Ruzzier; **LF** Lorenzo Fortini; **MA** Matteo Annessi; **MMA** Marilena Marconi; **MME** Maurizio Mei; **GP** Guido Pagliano; **MZUR3** "Museum of Zoology and Comparative Anatomy", Department of Science, Roma Tre University (Rome, Italy); **MZUR** "Museum of Zoology" of La Sapienza University of Rome (Rome, Italy).

Photographs were taken with Visionary Digital LK Lab System (Visionary Digital, Palmyra, VA) equipped with a Canon EOS 6D mark II dSLR camera and an MP-E 65 mm f/2.8 1–5× lens (Canon, Tokyo, Japan). This device automatically captured stacks of images taken at different focal planes and combined them using Helicon Focus 7 software. SEM images were obtained using a FIB/SEM Helios 5 CX DualBeam (Thermo Scientific, Brno, Czech Republic) at the LIME (Electron Microscopy Interdepartmental Laboratory, Rome, Italy) of Roma Tre University.

Morphological terms follow Michener (2007). The following abbreviations are used in the species description: A = antennal segments (counting from the scape), S = metasomal sterna, T = metasomal terga. The nomenclature is in accordance with Kuhlmann et al. (2018) and Ghisbain et al. (2023), while the systematic framework reported in Hymenoptera: Apoidea: Anthophila of Italy by Comba (2019) was followed for the subspecies *Andrena pilipes iliensis*.

Table 1. Material examined from the wild bee collections deposited in the "Museum of Zoology and Comparative Anatomy" of Roma Tre University and in the "Museum of Zoology" of La Sapienza University of Rome.

Taxon	Sex	Collection site	Date	Collector	Identifier	Depositories
Andrena pilipes f. *iliensis*	1♂	Italy (Sardinia)	27 Jun. – 1 Jul. 2016	MME	MME	MZUR
Andrena pilipes f. *iliensis*	1♀	Italy (Sardinia)	3 Apr. 2023	ADG & MA	MA	MZUR3
Andrena pilipes f. *iliensis*	2♀	Italy (Sardinia)	12 Apr. 2025	AR & DL	MA	MZUR3
Andrena pilipes s.s.	1♀	Italy (Sicilia)	25 Jun. 1977	GP	GP	MZUR
Andrena pilipes s.s.	1♂	Italy (Sicilia)	4 Jun. 1985	GP	GP	MZUR
Andrena pilipes s.s.	1♂, 1♀	Italy (Tuscany)	28–30 Apr. 2019	MME	MME	MZUR
Andrena pilipes s.s.	1♂	Italy (Veneto)	8 Jul. 2021	ER	MA	MZUR3
Andrena pilipes s.s.	3♀	Italy (Latium)	13–23 Apr. 2022	LF	MME	MZUR3
Andrena pilipes s.s.	2♀, 1♂	Italy (Latium)	26–28 Jun. 2023	MMA	MA	MZUR3
Andrena pilipes s.s.	4♀	Italy (Latium)	21 Mar. – 2 Apr. 2025	MA	MA	MZUR3

Results

Genus *Andrena* Fabricius, 1775
Subgenus *Plastandrena* Hedicke (1933)

Andrena culucciae **Annessi, Riccieri & Di Giulio, sp. nov**
https://zoobank.org/8521C5B4-D4F7-474A-A1CC-8E6CEB6C99E1

Material examined. Italy Sardinia, Culuccia Peninsula (Sassari Province) ***Holotype***: • 1 ♂; 41.194361°N, 9.288000°E; 18 May 2024; ADG & MA leg.; entomological nets; on *Armeria pungens* (Link) Hoffmanns. & Link (Plumbaginaceae) in herbaceous dune vegetation; MZUR3. ***Paratypes***: • 1 ♀; 41.199972°N, 9.292444°E; 17 May 2022; ADG & MA leg.; entomological nets; on *Cistus monspeliensis* L. (Cistaceae) in Mediterranean maquis; GenBank: PX058890; MZUR3. • 3 ♀; 41.194361°N, 9.288000°E; 18 May 2022; ADG & MA leg.; entomological nets; on *Echium plantagineum* L. and *Jasione montana* L. (Campanulaceae) in herbaceous dune vegetation; GenBank: PX058884; MZUR3. • 1 ♀; 41.203083°N, 9.290833°E; 01 Jun 2023; ADG & MA leg.; entomological nets; on *Bunias erucago* L. (Brassicaceae) in pasture; MZUR3. • 2 ♀; 41.194361°N, 9.288000°E; 01 June 2023; ADG & MA leg.; entomological nets; on *Armeria pungens* (Link) Hoffmanns. & Link (Plumbaginaceae) in herbaceous dune vegetation; MZUR3. • 37 ♀, 1 ♂; 41.194361°N, 9.288000°E; 18 May 2024; ADG & MA leg.; entomological nets; on *Armeria pungens* (Link) Hoffmanns. & Link (Plumbaginaceae) in herbaceous dune vegetation; GenBank: PX058885, PX058886, PX058887, PX058888, PX058889, PX058891; MZUR3.

Molecular evidence. The eight COI sequences obtained from our specimens had a total length of 570 bp and were monophyletic, with high support both in the NJ tree (bootstrap = 99) and in the ML tree (UFB = 99; SH-aLRT = 98.4) (Figs 2, 3). The phylogenetic relationships among *A. nigrospina*, *A. pilipes* and *A. culucciae* were clearer in the NJ tree, where *A. culucciae* forms a clade that is sister to *A. pilipes* + *A. nigrospina* (Fig. 2). In contrast, the ML tree does not recover *A. nigrospina* as a monophyletic group, preventing the resolution of relationships among the three taxa, even though *A. culucciae* emerges as an isolated and well-defined clade denoting genetic differentiation from *A. nigrospina* and *A. pilipes* (Fig. 3). P-distance values were as follows: *A. culucciae* vs *A. nigrospina* = 1.64%; *A. culucciae* vs *A. pilipes* = 1.74%; *A. pilipes* vs *A. nigrospina* = 1.01%. Relationships among haplotypes in the haplotype network (Fig. 4) were coherent with those emerging from the NJ and with the p-distance values. 16 haplotypes were detected: nine belonging to *Andrena pilipes*, one of which is shared with the subspecies *A. pilipes* f. *iliensis*; five belonging to *A. nigrospina*; and three belonging to *A. culucciae*.

Diagnosis. The new species *Andrena culucciae* can easily be recognized as a *Plastandrena* due to its distinctly widened and curved hind tibial spur at the base, the rugose areolate propodeal triangle, the rounded pronotum and coarsely punctate terga. It is structurally very similar to the other taxa within the *A. pilipes*/*nigrospina* complex because of its large size, dark body, dense network of raised rugosity on

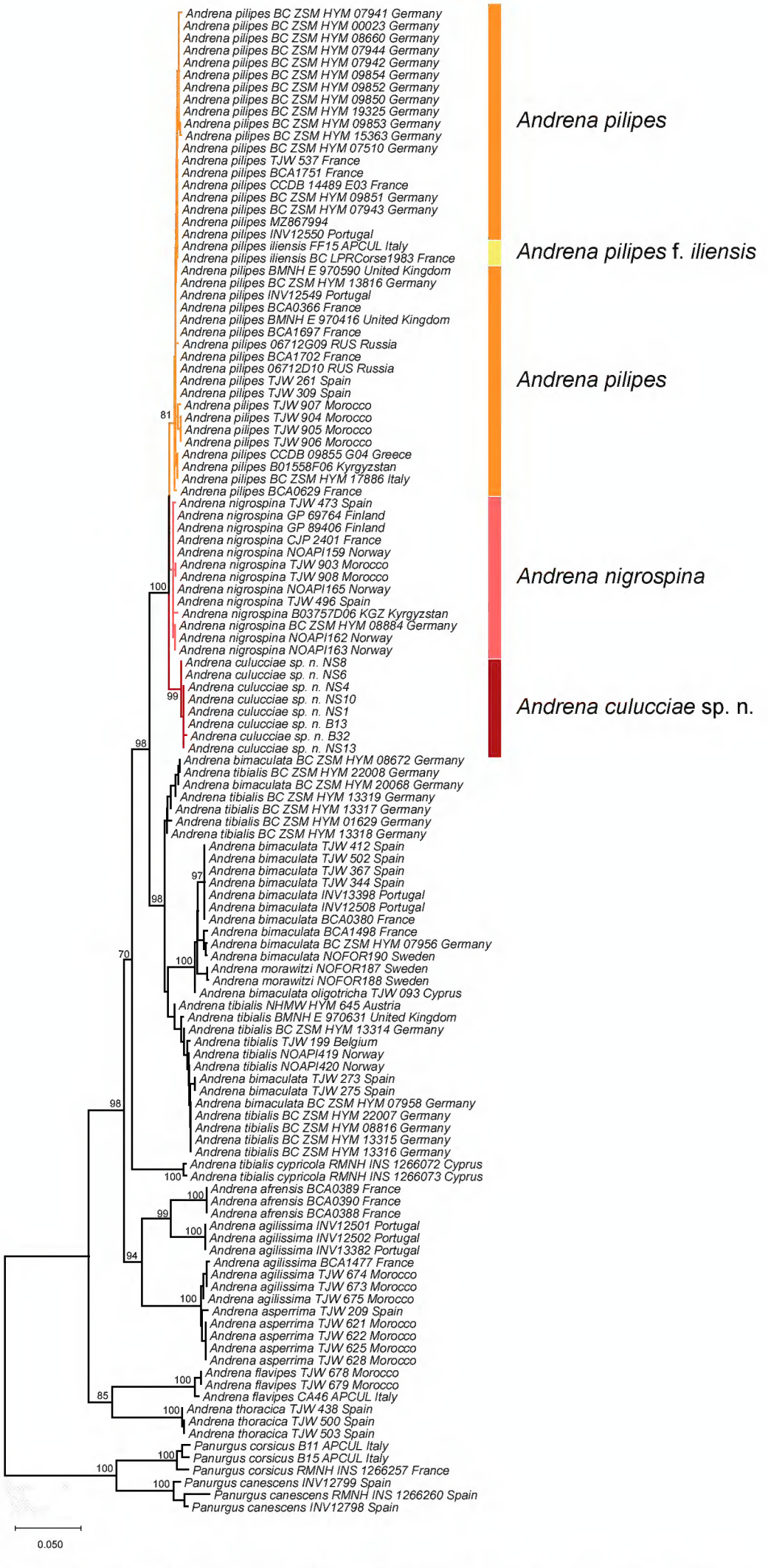
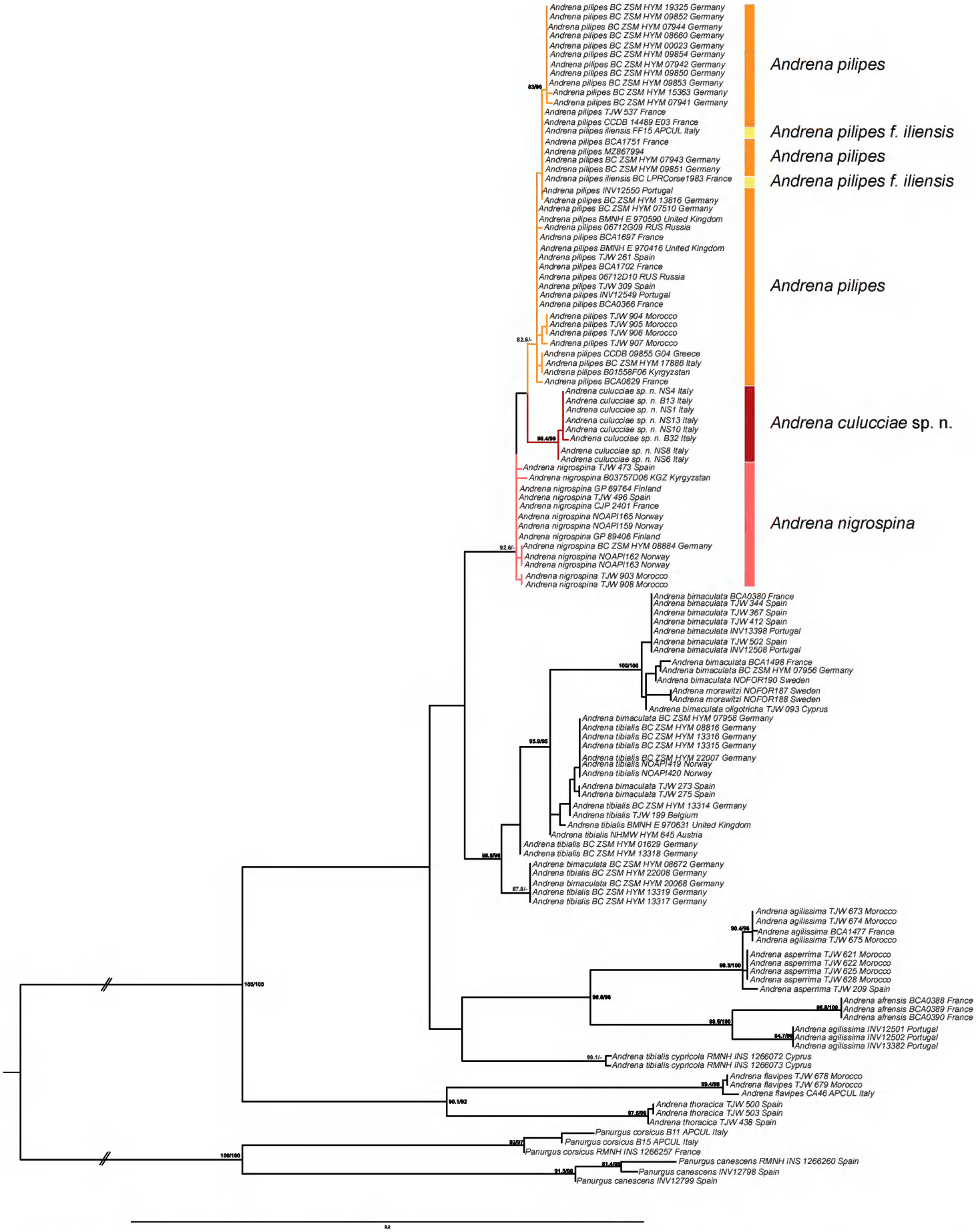

Andrena pilipes BC ZSM HYM 07941 Germany
Andrena pilipes BC ZSM HYM 00023 Germany
Andrena pilipes BC ZSM HYM 08660 Germany
Andrena pilipes BC ZSM HYM 07944 Germany
Andrena pilipes BC ZSM HYM 07942 Germany
Andrena pilipes BC ZSM HYM 09854 Germany
Andrena pilipes BC ZSM HYM 09852 Germany
Andrena pilipes BC ZSM HYM 09850 Germany
Andrena pilipes BC ZSM HYM 19325 Germany
Andrena pilipes BC ZSM HYM 09853 Germany
Andrena pilipes BC ZSM HYM 15363 Germany
Andrena pilipes BC ZSM HYM 07510 Germany
Andrena pilipes TJW 537 France
Andrena pilipes BCA1751 France
Andrena pilipes CCDB 14489 E03 France
Andrena pilipes BC ZSM HYM 09851 Germany
Andrena pilipes BC ZSM HYM 07943 Germany
Andrena pilipes MZ867994
Andrena pilipes INV12550 Portugal
Andrena pilipes iliensis FF15 APCUL Italy
Andrena pilipes iliensis BC LPRCorse1983 France
Andrena pilipes BMNH E 970590 United Kingdom
Andrena pilipes BC ZSM HYM 13816 Germany
Andrena pilipes INV12549 Portugal
Andrena pilipes BCA0366 France
Andrena pilipes BMNH E 970416 United Kingdom
Andrena pilipes BCA1697 France
Andrena pilipes 06712G09 RUS Russia
Andrena pilipes BCA1702 France
Andrena pilipes 06712D10 RUS Russia
Andrena pilipes TJW 261 Spain
Andrena pilipes TJW 309 Spain
Andrena pilipes TJW 907 Morocco
Andrena pilipes TJW 904 Morocco
Andrena pilipes TJW 905 Morocco
Andrena pilipes TJW 906 Morocco
Andrena pilipes CCDB 09855 G04 Greece
Andrena pilipes B01558F06 Kyrgyzstan
Andrena pilipes BC ZSM HYM 17886 Italy
Andrena pilipes BCA0629 France
Andrena nigrospina TJW 473 Spain
Andrena nigrospina GP 69764 Finland
Andrena nigrospina GP 89406 Finland
Andrena nigrospina CJP 2401 France
Andrena nigrospina NOAPI159 Norway
Andrena nigrospina TJW 903 Morocco
Andrena nigrospina TJW 908 Morocco
Andrena nigrospina NOAPI165 Norway
Andrena nigrospina TJW 496 Spain
Andrena nigrospina B03757D06 KGZ Kyrgyzstan
Andrena nigrospina BC ZSM HYM 08884 Germany
Andrena nigrospina NOAPI162 Norway
Andrena nigrospina NOAPI163 Norway
Andrena culucciae sp. n. NS8
Andrena culucciae sp. n. NS6
Andrena culucciae sp. n. NS4
Andrena culucciae sp. n. NS10
Andrena culucciae sp. n. NS1
Andrena culucciae sp. n. B13
Andrena culucciae sp. n. B32
Andrena culucciae sp. n. NS13
Andrena bimaculata BC ZSM HYM 08672 Germany
Andrena tibialis BC ZSM HYM 22008 Germany
Andrena bimaculata BC ZSM HYM 20068 Germany
Andrena tibialis BC ZSM HYM 13319 Germany
Andrena tibialis BC ZSM HYM 13317 Germany
Andrena tibialis BC ZSM HYM 01629 Germany
Andrena tibialis BC ZSM HYM 13318 Germany
Andrena bimaculata TJW 412 Spain
Andrena bimaculata TJW 502 Spain
Andrena bimaculata TJW 367 Spain
Andrena bimaculata TJW 344 Spain
Andrena bimaculata INV13398 Portugal
Andrena bimaculata INV12508 Portugal
Andrena bimaculata BCA0380 France
Andrena bimaculata BCA1498 France
Andrena bimaculata BC ZSM HYM 07956 Germany
Andrena bimaculata NOFOR190 Sweden
Andrena morawitzi NOFOR187 Sweden
Andrena morawitzi NOFOR188 Sweden
Andrena bimaculata oligotricha TJW 093 Cyprus
Andrena tibialis NHMW HYM 645 Austria
Andrena tibialis BMNH E 970631 United Kingdom
Andrena tibialis BC ZSM HYM 13314 Germany
Andrena tibialis TJW 199 Belgium
Andrena tibialis NOAPI419 Norway
Andrena tibialis NOAPI420 Norway
Andrena bimaculata TJW 273 Spain
Andrena bimaculata TJW 275 Spain
Andrena bimaculata BC ZSM HYM 07958 Germany
Andrena tibialis BC ZSM HYM 22007 Germany
Andrena tibialis BC ZSM HYM 08816 Germany
Andrena tibialis BC ZSM HYM 13315 Germany
Andrena tibialis BC ZSM HYM 13316 Germany
Andrena tibialis cypricola RMNH INS 1266072 Cyprus
Andrena tibialis cypricola RMNH INS 1266073 Cyprus
Andrena afrensis BCA0389 France
Andrena afrensis BCA0390 France
Andrena afrensis BCA0388 France
Andrena agilissima INV12501 Portugal
Andrena agilissima INV12502 Portugal
Andrena agilissima INV13382 Portugal
Andrena agilissima BCA1477 France
Andrena agilissima TJW 674 Morocco
Andrena agilissima TJW 673 Morocco
Andrena agilissima TJW 675 Morocco
Andrena asperrima TJW 209 Spain
Andrena asperrima TJW 621 Morocco
Andrena asperrima TJW 622 Morocco
Andrena asperrima TJW 625 Morocco
Andrena asperrima TJW 628 Morocco
Andrena flavipes TJW 678 Morocco
Andrena flavipes TJW 679 Morocco
Andrena flavipes CA46 APCUL Italy
Andrena thoracica TJW 438 Spain
Andrena thoracica TJW 500 Spain
Andrena thoracica TJW 503 Spain
Panurgus corsicus B11 APCUL Italy
Panurgus corsicus B15 APCUL Italy
Panurgus corsicus RMNH INS 1266257 France
Panurgus canescens INV12799 Spain
Panurgus canescens RMNH INS 1266260 Spain
Panurgus canescens INV12798 Spain
81
100
99
98
97
98
70
100
98
100
99
100
94
100
85
100
100
100
100
0.050
Andrena pilipes
Andrena pilipes f. iliensis
Andrena pilipes
Andrena nigrospina
Andrena culucciae sp. n.

Figure 2. Neighbor Joining Tree based on the COI highlighting the relationship between *Andrena pilipes*, *A. nigrospina* and *A. culucciae* sp. nov. within the context of the Western Palearctic *Plastandrena*. Numbers at nodes indicate bootstrap values; only values above 70 are represented.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Andrena pilipes BC ZSM HYM 00023 Germany
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Andrena pilipes BC ZSM HYM 07942 Germany
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Andrena pilipes iliensis BC LPRCorse1983 France
Andrena pilipes INV12550 Portugal
Andrena pilipes BC ZSM HYM 13816 Germany
Andrena pilipes BC ZSM HYM 07510 Germany
Andrena pilipes BMNH E 970590 United Kingdom
Andrena pilipes 06712G09 RUS Russia
Andrena pilipes BCA1697 France
Andrena pilipes BMNH E 970416 United Kingdom
Andrena pilipes TJW 261 Spain
Andrena pilipes BCA1702 France
Andrena pilipes 06712D10 RUS Russia
Andrena pilipes TJW 309 Spain
Andrena pilipes INV12549 Portugal
Andrena pilipes BCA0366 France
Andrena pilipes TJW 904 Morocco
Andrena pilipes TJW 905 Morocco
Andrena pilipes TJW 906 Morocco
Andrena pilipes TJW 907 Morocco
Andrena pilipes CCDB 09855 G04 Greece
Andrena pilipes BC ZSM HYM 17886 Italy
Andrena pilipes B01558F06 Kyrgyzstan
Andrena pilipes BCA0629 France
Andrena culucciae sp. n. NS4 Italy
Andrena culucciae sp. n. B13 Italy
Andrena culucciae sp. n. NS1 Italy
Andrena culucciae sp. n. NS13 Italy
Andrena culucciae sp. n. NS10 Italy
Andrena culucciae sp. n. B32 Italy
Andrena culucciae sp. n. NS8 Italy
Andrena culucciae sp. n. NS6 Italy
Andrena nigrospina TJW 473 Spain
Andrena nigrospina B03757D06 KGZ Kyrgyzstan
Andrena nigrospina GP 69764 Finland
Andrena nigrospina TJW 496 Spain
Andrena nigrospina CJP 2401 France
Andrena nigrospina NOAPI165 Norway
Andrena nigrospina NOAPI159 Norway
Andrena nigrospina GP 89406 Finland
Andrena nigrospina BC ZSM HYM 08884 Germany
Andrena nigrospina NOAPI162 Norway
Andrena nigrospina NOAPI163 Norway
Andrena nigrospina TJW 903 Morocco
Andrena nigrospina TJW 908 Morocco
Andrena pilipes
Andrena pilipes f. iliensis
Andrena pilipes
Andrena pilipes f. iliensis
Andrena pilipes
Andrena culucciae sp. n.
Andrena nigrospina
Andrena bimaculata BCA0380 France
Andrena bimaculata TJW 344 Spain
Andrena bimaculata TJW 367 Spain
Andrena bimaculata TJW 412 Spain
Andrena bimaculata INV13398 Portugal
Andrena bimaculata TJW 502 Spain
Andrena bimaculata INV12508 Portugal
Andrena bimaculata BCA1498 France
Andrena bimaculata BC ZSM HYM 07956 Germany
Andrena bimaculata NOFOR190 Sweden
Andrena morawitzi NOFOR187 Sweden
Andrena morawitzi NOFOR188 Sweden
Andrena bimaculata oligotricha TJW 093 Cyprus
Andrena bimaculata BC ZSM HYM 07958 Germany
Andrena tibialis BC ZSM HYM 08816 Germany
Andrena tibialis BC ZSM HYM 13316 Germany
Andrena tibialis BC ZSM HYM 13315 Germany
Andrena tibialis BC ZSM HYM 22007 Germany
Andrena tibialis NOAPI419 Norway
Andrena tibialis NOAPI420 Norway
Andrena bimaculata TJW 273 Spain
Andrena bimaculata TJW 275 Spain
Andrena tibialis BC ZSM HYM 13314 Germany
Andrena tibialis TJW 199 Belgium
Andrena tibialis BMNH E 970631 United Kingdom
Andrena tibialis NHMW HYM 645 Austria
Andrena tibialis BC ZSM HYM 01629 Germany
Andrena tibialis BC ZSM HYM 13318 Germany
Andrena bimaculata BC ZSM HYM 08672 Germany
Andrena tibialis BC ZSM HYM 22008 Germany
Andrena bimaculata BC ZSM HYM 20068 Germany
Andrena tibialis BC ZSM HYM 13319 Germany
Andrena tibialis BC ZSM HYM 13317 Germany
Andrena agilissima TJW 673 Morocco
Andrena agilissima TJW 674 Morocco
Andrena agilissima BCA1477 France
Andrena agilissima TJW 675 Morocco
Andrena asperrima TJW 621 Morocco
Andrena asperrima TJW 622 Morocco
Andrena asperrima TJW 625 Morocco
Andrena asperrima TJW 628 Morocco
Andrena asperrima TJW 209 Spain
Andrena afrensis BCA0388 France
Andrena afrensis BCA0389 France
Andrena afrensis BCA0390 France
Andrena agilissima INV12501 Portugal
Andrena agilissima INV12502 Portugal
Andrena agilissima INV13382 Portugal
Andrena tibialis cypricola RMNH INS 1266072 Cyprus
Andrena tibialis cypricola RMNH INS 1266073 Cyprus
Andrena flavipes TJW 678 Morocco
Andrena flavipes TJW 679 Morocco
Andrena flavipes CA46 APCUL Italy
Andrena thoracica TJW 500 Spain
Andrena thoracica TJW 503 Spain
Andrena thoracica TJW 438 Spain
Panurgus corsicus B11 APCUL Italy
Panurgus corsicus B15 APCUL Italy
Panurgus corsicus RMNH INS 1266257 France
Panurgus canescens RMNH INS 1266260 Spain
Panurgus canescens INV12798 Spain
Panurgus canescens INV12799 Spain
0.2

Figure 3. Maximum Likelihood Tree based on the COI highlighting the relationship between *Andrena pilipes*, *A. nigrospina* and *A. culucciae* sp. nov. within the context of the Western Palearctic *Plastandrena*. Numbers at nodes indicate SH-aLRT/UFB values; only values of SH-aLRT above 80 and of UFB above 95 are reported.

the dorsolateral faces of the propodeum and clypeus with large and dense punctations. *Andrena culucciae* differs mainly from *Andrena pilipes* s.s. and *A. pilipes* f. *iliensis* in the male sex by a longer genital capsule and broader penis valves at the

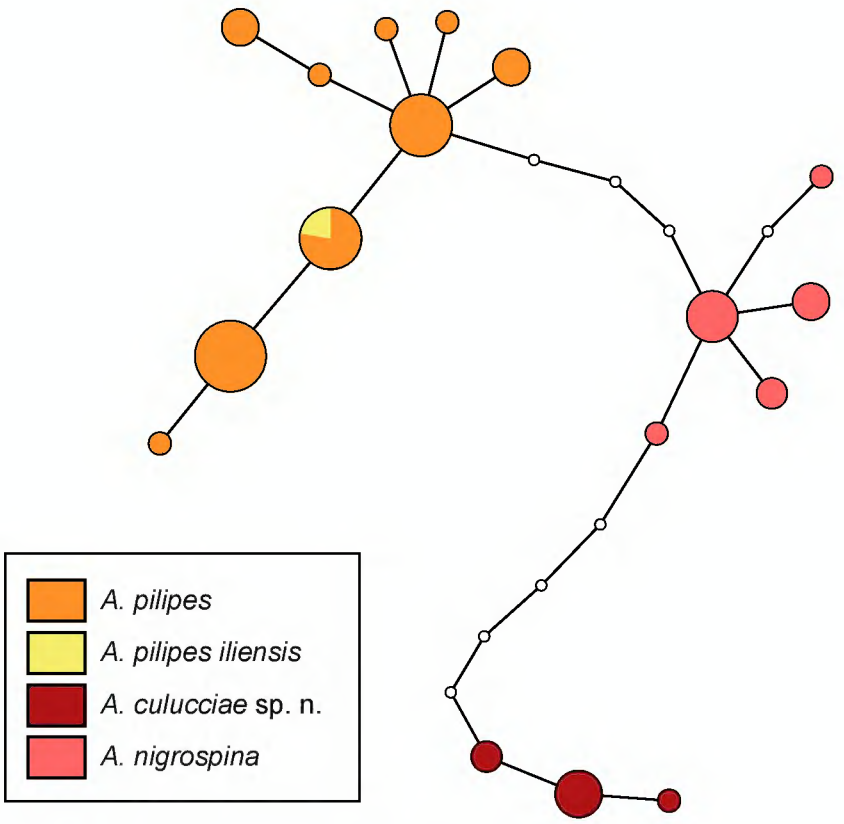

Figure 4. Haplotype network of *A. pilipes*, *A. nigrospina* and *A. culucciae* sp. nov. Colours assigned to each taxon are reported in the legend. Not sampled intermediate haplotypes are indicated by white dots.

base (Figs 5–7), as well as the translucent marginal zones of T2-T5 (Fig. 8), which also distinguishes it from *A. nigrospina*. Females can be distinguished based on the predominant coloration of the setae on the hind legs: in *A. culucciae*, the setae on the femur are white and the tibial scopa is brown, in *A. pilipes* f. *iliensis* both femoral setae and tibial scopa are black, while in *A. pilipes* and in *A. nigrospina* both are white (Fig. 9). Further morphological comparisons between *A. culucciae*, *A. pilipes* and *A. pilipes* f. *iliensis* are reported in Table 2.

Description. Male (Fig. 10). ***Body length*:** 12–13 mm. ***Head.*** Black, 1.2 times wider than long (Fig. 10B). Ocelloccipital distance slightly greater than lateral ocellar diameter. Paraocular areas depressed, both frons and paraocular areas with longitudinal wrinkles (Fig. 10B). Clypeus domed, slightly shiny with dense and large punctures, without an impunctate central line (Fig. 10B). Labral process trapezoidal, apex emarginate. Mandibles slightly crossing apically with sparse setae along outer margin, bidentate; apical tooth longer than preapical tooth; maxillary palpi with six segments, labial palpi with four segments. Gena moderately broad, about equal in width to compound eye. Vertex and face with whitish and brownish setae (Fig. 10B). Antenna dark, A3 slightly shorter than A4 (Fig. 10B). ***Mesosoma.*** Scutum and scutellum irregularly punctate, with punctures larger and sparser medially. Pronotum rounded laterally, without humeral angle. Episternum and lateral propodeal surface reticulate, propodeal triangle strongly carinate with internal surface rugose-areolate. Scutum and scutellum with moderately long whitish setae, episternum and propodeum with brownish setae (Fig. 10A). Legs dark, tarsal claw light brown, general pubescence dark brown, lighter on tarsal segments (Fig. 10A, C). Wings brownish, venation dark brown and

Table 2. Morphological differences between *Andrena culucciae* sp. nov., *A. pilipes* f. *iliensis* and *A. pilipes* s.s. *As no information is available in the literature, the measurements only refer to the characters of the material examined (see Table 1).

	***Andrena culucciae* sp. nov.**	***A. pilipes* f. *iliensis***	***Andrena pilipes* s.s.**
Male			
1) dimension	12–13 mm	11 mm*	13–15 mm
2) setae on face and vertex	whitish and brownish	dark brown and black	dark brown and black
3) length of A4 compared to A3	1	1,15*	1,20*
4) setae on mesosoma	whitish, brownish and greyish	brown and black	brown and black
5) marginal zones of T2-T5	translucent (Fig. 8A)	dark (Fig. 8B)	dark (Fig. 8C)
6) maximum width of penis valves (mm)	0,36–0,38	0,26*	0,25–0,27*
7) apex of penis valves	slender	inflated	inflated
8) cuspis (volsella)	triangular and spatulate, with a rounded apex (Fig. 11A,C)	–	short and slender, apically pointed and slightly curved (Fig. 11B,D)
Female			
1) dimension	14–16 mm	12–14 mm	14–16 mm
2) setae on face and the vertex	brown	black	black
3) fovea	brown	black	black
4) setae on hind femur	white (Fig. 9A)	black (Fig. 9B)	white (Fig. 9C)
5) tibial scopa	brown (Fig. 9A)	black (Fig. 9B)	white (Fig. 9C)

Figure 5. Dorsal view of male genitalia **A** *Andrena culucciae* sp. nov. **B** *Andrena pilipes* f. *iliensis* **C** *Andrena pilipes* s.s. Scale bars: 1 mm.

stigma light brown and nervulus slightly antefurcal; tegulae brown with surface rugose (Fig. 10A). ***Metasoma.*** Terga dark and regularly punctate, punctures separated on average by 2 puncture diameters (Fig. 10A). Disc of T1 with sparser punctation, marginal zones of T2-T5 slightly depressed and translucent (Fig. 10A). T1-T4 with very sparse short brown setae, last tergites with longer and darker hairs. Pygidial plate absent. S7 deeply emarginate apically with lateral lobes hairy. S8 hairy, slightly emarginate apically (Fig. 10F). Genitalia elongate, gonocoxae with clearly produced apical teeth, these diverging slightly; gonostyli with apical spatulate parts elongate and

Figure 6. SEM images of the dorsal view of male genitalia **A** *Andrena culucciae* sp. nov. **B** *Andrena pilipes* s.s. Scale bars: 0,5 mm.

parallel margins (Figs 6A, 7A, 10D, E). Penis valves broad at the base (Figs 6A, 7A, 10D), progressively tapering to the apex (Fig. 7A). Volsella transverse, with rounded outer margins, and with both cuspis and digitus well developed (Figs 7A, 11A). Digitus curved, subparallel to the cuspis, basoventrally fused with the basivolsella, and with a bulged apex (Figs 7A, 11A, C). Cuspis triangular and spatulate, with a rounded apex and numerous erect setae of different lengths (Fig. 11C). Cuneiform tooth-like cuticular structure, obliquely truncate and laterally directed, located in the ventral apical part of the digitus and the dorsal subapical part of the cuspis (Fig. 11C). Basivolsella bulged, with numerous elongate setae (approximately 20, Fig. 11A).

Female (Fig. 12). ***Body length*:** 14–16 mm. ***Head.*** Black, 1.2 times wider than long (Fig. 12B). Ocelloccipital distance slightly greater than lateral ocellar diameter. Fovea uniformly depressed along entire length, with lower margin ending distinctly below antennal insertion (Fig. 12B). Frons with longitudinal wrinkles (Fig. 12B). Clypeus, gena, maxillary and labial palpi as in male, labral process trapezoidal but without apical emargination. Mandibles slightly crossing apically with sparse setae along outer margin, unidentate. Subgenal coronet present and well-developed. Fovea, vertex and face with brown setae (Fig. 12B). Antenna dark, A3 slightly exceeds A4+5, shorter than A4+5+6 (Fig. 12B). ***Mesosoma.*** Scutum, scutellum, pronotum, episternum, propodeum and propodeal triangle as in male. Scutum and scutellum with moderately long black and dark brown setae, episternum and propodeum with brownish setae (Fig. 12A). Propodeal corbicula incomplete, possessing only a dorsal fringe; internal surface mostly glabrous,

Figure 7. Dissected genital capsule of **A** *Andrena culucciae* sp. nov. **B** *Andrena pilipes* s.s. From top to bottom: gonocoxae and gonostyli, penis valves and volsella. Scale bars: 1 mm.

with very sparse setae. Legs dark, with tarsal claw light brown; fore and middle legs with general pubescence dark brown, lighter on tarsal segments; hind legs with white hairs on femur and tibial scopa predominantly composed of brown hairs (Figs 9A, 12A, C). Wings and tegulae as in male but browner (Fig. 12A). ***Metasoma.*** Terga entirely dark,

Figure 8. Dorsal view of male tergita **A** *Andrena culucciae* sp. nov. **B** *Andrena pilipes* f. *iliensis* **C** *Andrena pilipes* s.s. Scale bars: 1 mm.

Figure 9. Hind legs of female **A** *Andrena culucciae* sp. nov. **B** *Andrena pilipes* f. *iliensis* **C** *Andrena pilipes* s.s. Scale bars: 1 mm.

punctation similar to that of the male (Fig. 12A). Pubescence of T1-T4 as in male, apical fringe of T5 and hairs flanking pygidial plate dark brown (Fig. 12A). Pygidial plate V-shaped with round apex and a distinct raised internal triangle; internal surface rough.

Derivatio nominis. We named the new species after Culuccia Peninsula (Sardinia, SS, Italy), where the specimens were collected.

Distribution. Sardinia (Italy).

Ecological aspects. Specimens of *Andrena culucciae* sp. nov. were mainly observed on *Armeria pungens* (Plumbaginaceae) in the herbaceous vegetation of coastal dunes with coarse sand (Fig. 13). However, this new taxon was also recorded on plant species belonging to different families, such as Boraginaceae, Brassicaceae, Campanulaceae and Cistaceae. These observations suggest that *A. culucciae* sp. nov. is polylectic, like its closest related taxa (Beil et al. 2008).

Figure 10. *Andrena culucciae* sp. nov. male **A** dorsal view **B** head **C** lateral view **D** genitalia dorsal view **E** genitalia profile **F** sternite 8. Scale bars: 5 mm (**A**); 2 mm (**B, C**); 1 mm (**D–F**)

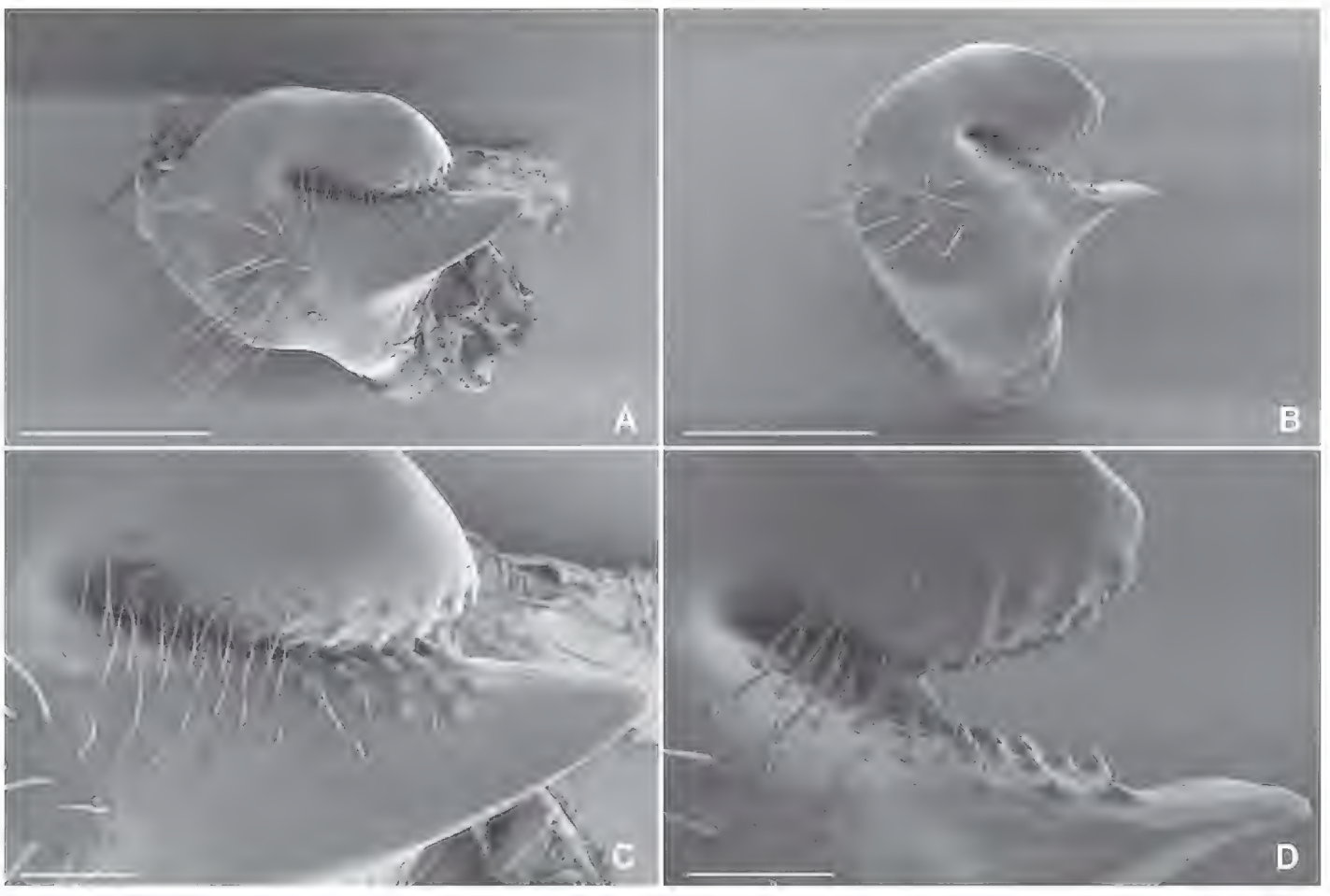

Figure 11. SEM images of the lateral view of the volsella of (**A, C**) *Andrena culucciae* sp. nov. and (**B, D**) *Andrena pilipes* s.s. (**A, B**) general view; (**C, D**) details of the apex of the cuspis and the digitus. Scale bars: 0,2 mm (**A, B**); 0,05 mm (**C, D**).

Figure 12. *Andrena culucciae* sp. nov. female **A** dorsal view **B** head **C** lateral view. Scale bars: 5 mm (**A**); 2 mm (**B, C**)

Figure 13. Herbaceous dune vegetation in the Culuccia Peninsula (Sardinia, Italy), where most of the *Andrena culucciae* sp. nov. specimens were collected (**A**) Female of *Andrena culucciae* sp. nov. on *Armeria pungens* (**B**) (Photos by M. Annessi).

Identification key to the *A. pilipes/nigrospina* complex

Female:

1	Tibial scopa black or predominantly composed of brown hairs **2**
–	Tibial scopa predominantly white (Fig. 9C) **3**
2	Tibial scopa predominantly composed of brown hairs (Fig. 9A). Fovea brown. Univoltine (May-early June). Larger, 14–16 mm ..... ***A. culucciae* sp. nov.**
–	Tibial scopa black (Fig. 9B). Fovea black. Bivoltine (April and late June-July). Smaller, 12–14 mm ..... ***A. pilipes* f. *iliensis***
3	Bivoltine (typically March-April and July-August) ..... ***A. pilipes***
–	Univoltine (typically May-June) ..... ***A. nigrospina***

Male:

1	Marginal zones of T2-T5 dark or translucent. Penis valves basally broad (Figs 5A, 6A, 7A) **2**
–	Marginal zones of T2-T5 dark. Penis valves basally narrow (Figs 5B, C, 6B, 7B) **3**
2	Marginal zones of T2-T5 dark (cfr. Fig. 8B, C) ..... ***A. nigrospina***
–	Marginal zones of T2-T5 translucent (Fig. 8A) ..... ***A. culucciae* sp. nov.**
3	Larger, 13–15 mm ..... ***A. pilipes***
–	Smaller, 11 mm ..... ***A. pilipes* f. *iliensis***

Conclusions

In this study, we describe a new species within the *Andrena pilipes/nigrospina* complex. The new species is supported by several diagnostic morphological characters. In particular, the structure of the genital capsule and the translucent marginal zones of the tergites enable reliable identification of male individuals. Moreover, females of *A. culucciae* sp. nov. exhibit distinct and unambiguous coloration patterns of setae, in contrast to *A. pilipes* and *A. nigrospina*, whose females cannot be reliably separated based on pubescence color (Wood 2023a).

COI data is not conflicting with the morphological evidence, but they are not sufficient to resolve the relationships within the *Andrena pilipes/nigrospina* complex and to clearly delimit the three species. This is not surprising in this group (Wood 2023b) or in other insects (e.g. in Orthoptera: Hochkirch et al. 2024; Nabholz et al. 2024; in beetles: Poloni et al. 2023; Riccieri et al. 2024), and the application of more advanced genetic techniques would be particularly beneficial, especially in cases where greater ambiguity exists between morphological data and standard barcodes. However, here we combined significant morphological evidences with molecular results that still point to the uniqueness of our specimens: they consistently form a well-supported, isolated clade in both phylogenetic trees (Figs 2, 3); the p-distances between *A. culucciae* and both *A. nigrospina* and *A. pilipes* (1.64% and 1.74%) are greater than that between *A. nigrospina* and *A. pilipes* (1.01%); and the haplotype network shows clear genetic separation among the three species' haplotypes.

Phenological observations also support the distinction between the new species and the syntopic *A. pilipes*. *Andrena culucciae* sp. nov. was observed only in May and early June, suggesting a univoltine life cycle, with a flight period that does not overlap with that of *A. pilipes*, which occurs in early spring and summer. This suggests that *A. culucciae* sp. nov. has flight patterns more similar to *A. nigrospina*, the univoltine species absent in Italy, which also flies in May and June. This hypothesis is supported by the comprehensive survey of wild bees conducted on the Culuccia Peninsula (Annessi et al. 2025a), in which *A. pilipes* f. *iliensis* was recorded in April without overlapping with the new taxon.

It is worth notice that *Andrena pilipes* f. *iliensis* specimens were completely nested within the clade of *A. pilipes* s.s. in the NJ and ML trees (Figs 2, 3) and shared the same haplotype with other specimens of *A. pilipes* s.s. (Fig. 4). Moreover, this subspecies shows no significant morphological differences from *A. pilipes* s.s., except for smaller body size and the black scopa, which could be attributed to "warm forms" occurring in warmer regions (Baker 1994; Grünwaldt 1976). According to these results and observations, we suggest that *Andrena pilipes iliensis* is not a valid subspecies, but rather an insular form adapted to a restricted habitat due to its small dimensions, as hypothesized by Baker (1994).

Finally, more observations and molecular studies could confirm the distribution of this new taxon, which could be endemic to Sardinia, or Sardinia-Corsica, but also present in other areas of the Mediterranean. Further studies are needed to investigate the association of *Andrena culucciae* sp. nov. with coastal dune habitats, as this could have important implications for the conservation of the species, given the high vulnerability of these environments in the Mediterranean region (Zivkovic et al. 2017).

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Supplementary material 1

List of analyzed specimens whose COI sequences

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Data type: xlsx

Explanation note: The list were downloaded from BOLD or newly produced (*Andrena culucciae* sp. nov.).

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